METHOD AND APPARATUS FOR ANALYSING A LIQUID SAMPLE

Patent number: WO9518962
Publication date: 1995-07-13
Inventor: OEHLIN ERIK (SE)
Applicant: SWELAB INSTRUMENT AB (SE); OEHLIN ERIK (SE)
Classification:
- international: ***G01N1/38;*** *G01N1/00; G01N15/12;* **G01N1/38;** G01N1/00; G01N15/10; (IPC1-7): G01N1/38
- european: G01N1/38
Application number: WO1995SE00012 19950110
Priority number(s): SE19940000032 19940110

Also published as:
EP0739478 (A1)
US5731211 (A1)
EP0739478 (B1)
SE9400032 (L)
SE513881 (C2)
CN1138376 (C)

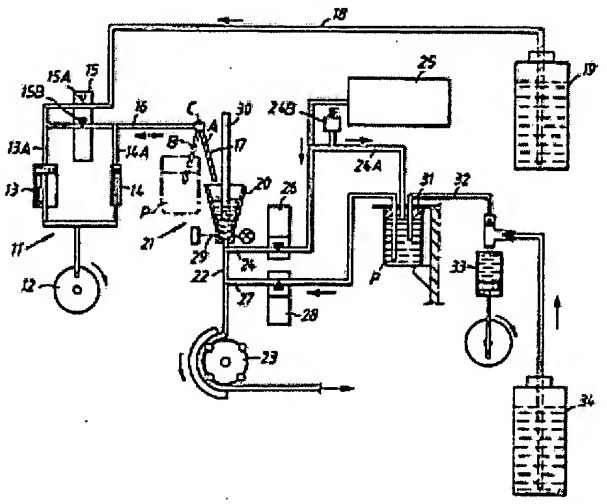
less <<

Cited documents:
DE3335641
EP0081919
EP0089937

Report a data error here

Abstract of **WO9518962**

In a method and apparatus for analysing a liquid sample, particularly a sample of blood, in different concentrations, a portion of a sample contained in a sample receptacle (P) and diluted to a predetermined concentration is again diluted to a predetermined lower concentration when it is transferred to a receiving vessel (20) in which the sample is analysed in respect of one or more sample parameters. The transfer is effected by means of a pipette (17) which in a first position (B) thereof is brought together with the sample receptacle (P) for aspiration of said sample portion and which is then shifted to a second position (A) for dispensing said portion and liquid diluent to the receiving vessel (20). The sample receptacle is then brought together with means (33, 25) for adding a predetermined quantity of liquid to the remainder of the sample in the sample receptacle (P) and for subsequent transfer of the sample from the sample receptacle to the receiving vessel (20) in which the sample is analysed in respect of one or more additional parameters.



Family list
13 family members for:
CN1138376
Derived from 10 applications.

Back to CN1138376

1 **Method and apparatus for analysing a liquid sample**
Publication info: **AU1469395 A** - 1995-08-01

2 **Method and apparatus for analysing a liquid sample**
Publication info: **CN1046583C C** - 1999-11-17
CN1138376 A - 1996-12-18

3 **Method and apparatus for analysing a liquid sample**
Publication info: **DE69524732D D1** - 2002-01-31

4 **Method and apparatus for analysing a liquid sample**
Publication info: **DE69524732T T2** - 2002-11-21

5 **METHOD AND APPARATUS FOR ANALYSING A LIQUID SAMPLE**
Publication info: **EP0739478 A1** - 1996-10-30
EP0739478 B1 - 2001-12-19

6 **Method and apparatus for analysing a liquid sample**
Publication info: **JP9507297T T** - 1997-07-22

7 **Method and apparatus for analysing a liquid sample**
Publication info: **SE513881 C2** - 2000-11-20

8 **Method and apparatus for analysing a liquid sample**
Publication info: **SE9400032 A** - 1995-07-11
SE9400032D D0 - 1994-01-10

9 **Method and apparatus for analysing a liquid sample**
Publication info: **US5731211 A** - 1998-03-24

10 **METHOD AND APPARATUS FOR ANALYSING A LIQUID SAMPLE**
Publication info: **WO9518962 A1** - 1995-07-13

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : G01N 1/38	A1	(11) International Publication Number: **WO 95/18962** (43) International Publication Date: 13 July 1995 (13.07.95)

(21) International Application Number: PCT/SE95/00012

(22) International Filing Date: 10 January 1995 (10.01.95)

(30) Priority Data:
9400032-0 10 January 1994 (10.01.94) SE

(71) Applicant *(for all designated States except US)*: SWELAB INSTRUMENT AB [SE/SE]; P.O. Box 839, S-120 03 Årsta (SE).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: ÖHLIN, Erik [SE/SE]; Krusbärsstigen 6, S-182 75 Stocksund (SE).

(74) Agent: NYBERG, Bengt; Carminger, Uusitalo & Nyberg Patentbyrå AB, P.O. Box 7274, S-103 89 Stockholm (SE).

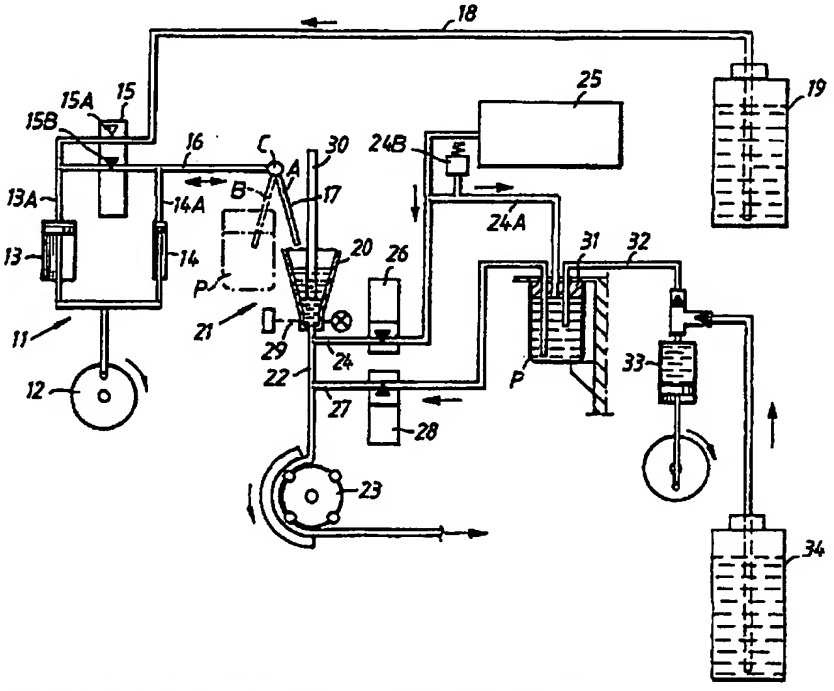
(81) Designated States: AU, CA, CN, FI, JP, KR, NO, RU, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: METHOD AND APPARATUS FOR ANALYSING A LIQUID SAMPLE

(57) Abstract

In a method and apparatus for analysing a liquid sample, particularly a sample of blood, in different concentrations, a portion of a sample contained in a sample receptacle (P) and diluted to a predetermined concentration is again diluted to a predetermined lower concentration when it is transferred to a receiving vessel (20) in which the sample is analysed in respect of one or more sample parameters. The transfer is effected by means of a pipette (17) which in a first position (B) thereof is brought together with the sample receptacle (P) for aspiration of said sample portion and which is then shifted to a second position (A) for dispensing said portion and liquid diluent to the receiving vessel (20). The sample receptacle is then brought together with means (33, 25) for adding a predetermined quantity of liquid to the remainder of the sample in the sample receptacle (P) and for subsequent transfer of the sample from the sample receptacle to the receiving vessel (20) in which the sample is analysed in respect of one or more additional parameters.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgystan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sodan
CG	Congo			SE	Sweden
CH	Switzerland	KR	Republic of Korea	SI	Slovenia
CI	Côte d'Ivoire	KZ	Kazakhstan	SK	Slovakia
CM	Cameroon	LI	Liechtenstein	SN	Senegal
CN	China	LK	Sri Lanka	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TG	Togo
CZ	Czech Republic	LV	Latvia	TJ	Tajikistan
DE	Germany	MC	Monaco	TT	Trinidad and Tobago
DK	Denmark	MD	Republic of Moldova	UA	Ukraine
ES	Spain	MG	Madagascar	US	United States of America
FI	Finland	ML	Mali	UZ	Uzbekistan
FR	France	MN	Mongolia	VN	Viet Nam
GA	Gabon				

Method and apparatus for analysing a liquid sample

This invention relates to a method for analysing a liquid sample, preferably in different dilutions, in which a sample contained in a sample receptacle is diluted to a predetermined concentration.

When testing blood in respect of different characteristic parameters or properties it is common practice to prepare from a sample of whole blood two or more subsamples which are diluted to different predetermined dilutions or concentrations. A test which requires dilution to at least two widely differing concentrations may be directed, for example, to determinations of parameters of red blood cells and blood platelets carried out on a subsample diluted to a first, low dilution (high concentration) and to determinations of parameters of white blood cells and haemoglobin carried out on a subsample diluted to a second, substantially higher dilution (lower concentration).

Generally, the subsamples are prepared by first diluting the sample of whole blood, or a portion thereof, to the low dilution in a first vessel for forming a first subsample. A portion of the first subsample is transferred to a second vessel together with a predetermined volume, e.g. 200 times the volume of the transferred subsample portion, of a diluent for forming a second subsample. An example of such serial dilution and apparatus for performing it is disclosed in US-A-4 746 491.

An object of the invention is to provide a method of the nature indicated above which can be performed rapidly and by simple apparatus in an automated analyser.

To this end, there is provided according to the invention a method and apparatus having the characterising features set forth in the independent claims. The dependent claims define preferred embodiments of the method and apparatus.

The invention will be described in greater detail below with reference to the accompanying diagrammatic drawing which illustrates an example of an apparatus for carrying out an embodiment of the method according to the invention.

The analyser illustrated diagrammatically in the drawing is adapted for serial examination of blood samples, namely for analysing each blood sample in respect of the number of red blood cells, the number of white blood cells and the number of platelets per unit volume of the sample and also the haemoglobin content and additional characteristic parameters or properties.

The analyser comprises a dual piston pump, generally designated by 11, which comprises a drive mechanism 12 driving a pair of piston pumps 13 and 14 serving as volume metering devices, and a valve mechanism 15. Piston pump 13 comprises a common inlet and outlet passage 13A and has a stroke volume which is substantially greater, 200 times greater, than the stroke volume of piston pump 14, which likewise comprises a common inlet and outlet passage 14A.

By way of a common conduit 16 the inlet-outlet passages 13A and 14A of both pumps 13, 14 are connected with one end of a pipette tube 17. A supply conduit 18, which can be blocked by means of a valve 15A of valve device 15, extends from a supply container 19 for isotonic diluent to the inlet-outlet passage 13A of pump 14. A section of the common conduit 16 extends between inlet-outlet passage 13A and inlet-outlet passage 14A and can be blocked by means of a valve 15B of valve device 15.

Pipette tube 17 is mounted on the body or housing (not shown) of the analyser such that it can take either of two predetermined positions, namely a position for aspirating a sample from a sample receptacle and a position for dispensing the aspirated sample to a receiving vessel together with a liquid diluent as will be described below. In the drawing, the pipette tube 17 is shown in full lines in the dispensing position at A, while it is shown in phantom lines in the aspirating position at B. A beaker-type sample receptacle P, into which the free end of the pipette tube 17 extends, is also indicated in phantom lines at the aspirating position. Shifting of the pipette tube 17 between positions A and B takes place by pivotal movement about a horizontal axis C and can be effected manually or automatically.

The receiving vessel, which is designated by 20, is open at the top and positioned in relation to the pipette tube 17 such that in the dispensing position A the free end of the pipette tube is above or in the receiving vessel. Receiving vessel 20 forms part of an instrument, generally designated by 21, which operates in well-known manner to analyse blood in respect of the number of red and white blood cells and platelets per unit volume of blood, the size distribution of the blood cells, the haematocrit and the haemoglobin content of the blood and other characteristic parameters or properties. This instrument may be, for example, the instrument which is produced and marketed under the designation SWELAB AutoCounter AC900 series by the assignee of the present invention.

At the bottom thereof, receiving vessel 20 is connected to a waste conduit 22 extending to a drain pump 23, to an air conduit 25, which extends to a pulsating air pump 25 and can be blocked by means of a valve 26, and to a liquid supply conduit 27, which can be blocked by means of a valve 28.

The lower portion of receiving vessel 20 forms a cuvette of a photometric haemoglobinometer which is diagrammatically indicated at 29 and is incorporated in instrument 21.

A measuring tube 30 of well-known type, which also forms part of instrument 21 and is used for counting and other examination of blood cells in accordance with conventional techniques, extends into the receiving vessel 20. In the wall thereof, measuring tube 30 has a microscopic measuring aperture through which a heavily diluted suspension of blood cells is passed during the examination. The impedance changes occurring across the measuring aperture when blood cells pass through it are detected and result in electric impulses the number and amplitudes of which are representative of the number and volume of the blood cells.

A beaker mount 31, suitably provided externally on the analyser body or housing (not shown) is adapted to hold and seal the beaker-like sample receptacle P. Through the portion of the beaker mount 31 which serves to seal the sample receptacle P extend the above-mentioned liquid supply conduit 27,

the inlet end of which is positioned near the bottom of the sample receptacle when the latter is held by the beaker mount, a branch 24A of the air conduit 24, and a further conduit 32 leading from a dispensing device 33 for a liquid reagent that haemolyses red blood cells. Dispensing device 33 is a piston pump device of known construction, the inlet conduit of which is connected to a reagent supply container 34.

The analysis of a blood sample in accordance with the method of the invention and using the above-described apparatus will now be described in greater detail. Starting point for the description is the situation in which the analysis of a preceding sample is completed and the sample has been removed from receiving vessel 20 which, however, still contains rinsing liquid.

From the sample of blood to be analysed there is initially prepared a diluted sample (dilution 1:200, for example) in a sample receptacle P. This preparation may be carried out in different ways, e.g. when drawing the sample by introducing a predetermined, precisely measured quantity of the blood in a sample receptacle which has been factory-filled with a predetermined quantity (4 ml, for example) of isotonic solution under sterile conditions and sealed such that the sterility is preserved during storage.

Pipette tube 17, which is initially in the dispensing position A, i.e. the position shown in full lines in the drawing, is shifted to the aspirating position B, which is indicated in phantom lines and in which it is readily accessible exteriorly of the analyser housing. Sample receptacle P is positioned beneath the free lower end of the pipette tube 17 and moved upwardly until the pipette tube dips into the diluted sample.

Double pump 11 is operated with valve 15A open and valve 15B closed and pump 13 draws 4 ml isotonic diluent through conduit 18 while pump 14 draws 20 μl of the diluted sample from sample receptacle P into pipette tube 17. As the aspirated sample volume is extremely small, it fills only a very short length, one or a few centimetres, of the pipette tube.

Sample receptacle P is then attached to beaker mount 31 as shown in full lines to the right in the drawing. Moreover, pipette tube 17 is manually or automatically returned to dispensing position A.

Simultaneously with the drawing of the sample into pipette tube 17 pump 23 is operated with valves 26 and 28 closed so that receiving vessel 20 is emptied of the rinsing liquid that remains from the preceding analysing cycle.

The analyser is then ready for carrying out the remaining part of the analysing process in response to a start signal.

When the start signal is given, double pump 11 dispenses the aspirated sample and the aspirated volume of diluent through pipette tube 17 to receiving vessel 20. Thus, the subsample then held by the receiving vessel will be diluted to 1:40000.

At the same time, dispensing device 33 feeds a predetermined volume, 4 ml for example, of the haemolysing agent through conduit 32 to the remaining portion of the prediluted sample in sample receptacle P. Moreover, air pump 25 is operated with valve 26 open to feed, through conduit 24, air pulses into receiving vessel 20 near the bottom thereof. Air is vented from sample receptacle P through branch conduit 24A and a valve 24B the opening pressure of which is set to a suitable value so that the pressure within sample receptacle P is limited to a predetermined value.

The subsample held in receiving vessel 20, which is diluted to the predetermined higher dilution, is analysed by means of the measuring tube 30 in respect of the number of white blood cells and platelets per unit volume of blood, the size and size distribution of the blood cells or other parameters as desired or required. Thereupon receiving vessel 20 is drained through conduit 22 by means of pump 23.

When receiving vessel 20 is emptied, all or a portion of the haemolysed subsample in sample container P is transferred through conduit 27 to receiving vessel 20 by means of air pump 25. Valve 26 then is in closed position so that the air from the pump is caused to flow through branch conduit 24A

into sample receptacle P. The opening pressure of valve 24B and the rate of air flow from pump 25 are set such that the transfer takes place rapidly.

The subsample now held in receiving vessel 20, which is diluted to a predetermined lower dilution, 1:400 for example, is analysed in respect of haemoglobin content, number of white blood cell per unit volume of blood etc., as desired or required.

Then the analysed subsample in receiving vessel 20 is removed through conduit 22 by means of pump 23 whereupon a predetermined quantity of diluent from supply container 19 is introduced into receiving vessel 20 by means of double pump 11. After this diluent has been removed in the same manner as the subsample, a predetermined quantity of diluent is again introduced by means of double pump 11. This diluent is allowed to remain in receiving vessel 20 pending the analysis of the next sample.

As will be understood from the preceding description of an embodiment of the method and apparatus according to the invention, the analysis can be carried out serially with short analysing cycles and using simple means. As soon as the sample has been aspirated from the sample receptacle P, the preparation of the subsample of the lower dilution may begin so that this subsample is ready for analysis as soon as the analysis of the subsample of the higher dilution is completed.

The use of a prediluted sample which is fed into the analyser instead of a sample of undiluted or whole blood minimizes the problems relating to carry-over from one sample to the next.

Naturally, it is within the scope of the invention to automate the method and apparatus to a higher degree than described above with reference to the illustrated exemplary embodiment. For example, the sample receptacles may be fed into and removed from the analyser by automatic means.

The invention is not limited to the multiple dilution which is carried out in the exemplary embodiment. It is

within the scope of the invention to carry out the analysis only on the portion of the sample which is diluted only once.

In the embodiment of the invention which is specifically illustrated in the drawing and described above, the relative movements of pipette tube 17 and receiving vessel 20 between the positions for aspirating a predetermined sample volume from sample receptacle P and dispensing this sample volume into receiving vessel 20 are brought about by moving the pipette tube while keeping the receiving vessel stationary. This is the arrangement that is normally preferred, but it is of course within the scope of the invention to arrange for the pipette tube to be stationary and instead arrange for the receiving vessel to move.

Claims

1. A method for analysing a liquid sample, particularly a sample of blood, in which a sample contained in a sample receptacle is diluted to a predetermined concentration, **characterised** in that

- a subsample comprising a predetermined volume of the sample contained in the sample receptacle (P) is aspirated into a pipette (17) with the pipette in a first position (B),

- the pipette (17) is positioned in a second position (A) in which the mouth of the pipette is located in or above a receiving vessel (20),

- the subsample aspirated into the pipette (17) is dispensed into the receiving vessel (20) together with a predetermined volume of liquid diluent, and

- the diluted subsample in the receiving vessel (20) is analysed in respect of at least one sample parameter.

2. A method according to claim 1 in which a portion of a sample contained in the sample receptacle and diluted to a first predetermined concentration is further diluted to a second predetermined concentration, **characterised** in that

- following the aspiration into the pipette (17) of the subsample, the sample receptacle (P) is brought together with a liquid dispensing device (33),

- a predetermined volume of liquid is added to the remainder of the sample in the sample receptacle by means of the dispensing device (33), and

- following removal of the diluted subsample from the receiving vessel (20) at least a portion of the further diluted sample in the sample receptacle (P) is transferred from the sample receptacle (P) to the receiving vessel (20) and analysed therein with respect to at least one further sample parameter.

3. A method according to claim 1 or 2, **characterised** in that the liquid added to the remainder of the sample in the sample receptacle (P) by means of the liquid dispensing device (33) is a reagent, such as a haemolysing agent.

4. A method according to claim 2 or 3, **characterised** in that the transfer of said sample portion from the sample

receptacle (P) to the receiving vessel (20) is effected by pressurising the sample receptacle (P).

5. A method according to any one of claims 1-4, **characterised** in that the diluted sample in the receiving vessel (20) is stirred by introducing air in the sample.

6. Apparatus for analysing a liquid sample, particularly a sample of blood, comprising

a sample aspirating pipette (17) having a sample intake end,

an aspirating device (11) connected with the sample aspirating pipette for aspirating a sample through the sample intake end and dispensing a predetermined volume of the sample and a predetermined volume of liquid diluent through the sample intake end into a receiving vessel (20), and

analysing means (30, 29) associated with the receiving vessel (20) for analysing the diluted portion of the sample in the receiving vessel (20) in respect of at least one sample parameter,

characterised in that

the sample aspirating pipette (17) is shiftable between a first position (B) for bringing the sample intake end of the pipette together with the sample in a sample receptacle (P) containing a sample to be aspirated and a second position (A) in which the sample intake end is above or in the receiving vessel (20).

7. Apparatus according to claim 6, **characterised** by

a connecting means (31) adapted to receive the sample receptacle (P) and connected with a dispensing device (33) for introducing a predetermined volume of liquid in the sample receptacle (P) and with means (25) for transferring at least a portion of the contents of the sample receptacle (P) to the receiving vessel (20), and

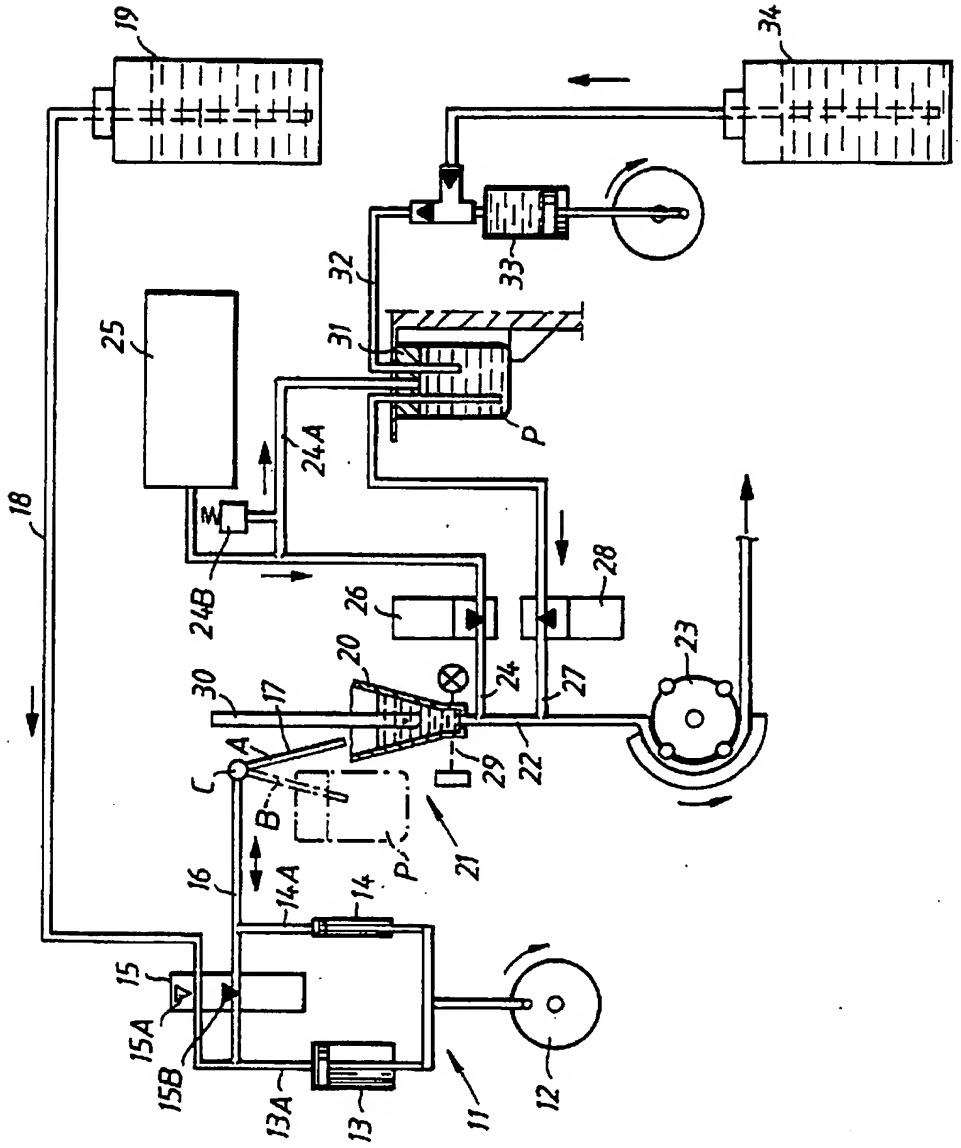
an analysing means (30, 29) associated with the receiving vessel (20) for analysing the sample transferred from the sample receptacle (P) to the receiving vessel (20) in respect of a second sample parameter.

8. Apparatus according to claim 7, **characterised** in that the connecting means (33) is adapted to sealingly engage the upper end of the sample receptacle (P).

9. Apparatus according to claim 7 or 8, **characterised** in that the connecting means (31) is connected with a device (35) for pressurising the interior of the sample receptacle (P) by feeding air into it.

10. Apparatus according to any one of claims 6 to 9, **characterised** by a device (25) for feeding air into the receiving vessel (20) adjacent the bottom thereof.

11. Apparatus according to any one of claims 6 to 10, **characterised** in that the pipette (17) is pivotally movable between the first position (B) and the second position (A).


19
34
32
33
31
25
24A
P
18
24B
26
28
20
30
17
24
27
23
A
C
B
29
22
21
16
14A
14
15
15A
15B
13A
13
11
12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 95/00012

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: G01N 1/38
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE, C2, 3335641 (SHIMADZU CORP.), 6 May 1993 (06.05.93), column 6, line 62 - column 8, line 13	1,6
A	EP, A1, 0081919 (OLYMPUS OPTICAL CO., LTD.), 22 June 1983 (22.06.83), abstract	1-11
A	EP, A1, 0089937 (ÖHLIN, ERIK), 28 Sept 1983 (28.09.83), page 1, line 6 - page 2, line 19	1-11

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 April 1995	25 -04- 1995
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Ulla Granlund Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

Information on patent family members

25/02/95

International application No.
PCT/SE 95/00012

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE-C2- 3335641	06/05/93	GB-A,B- 2132346	04/07/84
		JP-C- 1853789	07/07/94
		JP-B- 3058076	04/09/91
		JP-A- 59070994	21/04/84
EP-A1- 0081919	22/06/83	JP-A- 58086463	24/05/83
		US-A- 4522493	11/06/85
EP-A1- 0089937	28/09/83	SE-T3- 0089937	
		SE-B,C- 451164	07/09/87
		SE-A- 8201791	23/09/83
		US-A- 4746491	24/05/88